10/018700

INVESTOR IN PEOPLE

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

REC'D 16 AUG 2000

WIPO PCT

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 14 June 2000

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

1 JUL 1999

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1.	Your Reference	MLR/PG3692
2.	Patent application number *(The Patent office will fill in this part)*	01 JUL 1999 **9915413.0**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	GLAXO GROUP LIMITED GLAXO WELLCOME HOUSE BERKELEY AVENUE GREENFORD MIDDLESEX UB6 ONN GB
	Patents ADP number *(if you know it)*	07553003
	If the applicant is a corporate body, give the country/state of its corporation	GB
4	Title of the invention	PROPAGATION METHOD
5	Name of your agent *(if you know one)*	MARION L REES (SEE CONTINUATION SHEET)
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	GLAXO WELLCOME PLC GLAXO WELLCOME HOUSE, BERKELEY AVENUE GREENFORD, MIDDLESEX UB6 ONN, GB
	Patents ADP number *(if you know it)*	07553001

		Country	Priority application number *(if you know it)*	Date of Filing (day / month / year)
	If you are declaring priority from one or more earlier patent applications, give the country and date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing (day / month / year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8.	Is a statement of inventorship and of right to grant a patent required in support of this request? *(Answer yes if* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.*	YES

See note (d))

Pa[illegible]nts Form 1/77

Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	1
Description	12
Claim(s)	2
Abstract	-
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority Documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patent Form 9/77)*	
Request for substantive examination *(Patent Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature MARION L REES 01 July 1999

AGENT FOR THE APPLICANTS

12. Name and daytime telephone number of person to contact in the United Kingdom

CATE WEST

0181-966 8685

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication of communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the patent Act 1977 stops you from applying for a patent abroad without first getting written permission form the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been received

a) *Notes*

If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

If you have answered "Yes" Patents Form 7/77 will need to be filed.

d) *Once you have filled in the form you must remember to sign and date it.*

e) *For details of the fee and ways to pay please contact the Patent Office.*

Additional Agents
(See Page 1 No. 5)

NAME(S)	Alan HESKETH William Michael DADSON Michael ATKINSON Karen CRAWLEY Peter I. DOLTON Hugh B. DAWSON Wendy Anne FILLER Ruth Elizabeth HACKETT Catriona MacLeod HAMMER Audrey HAMMETT Graham M.H. LANE Stephanie Anne LEAROYD Helen Kaye QUILLIN Michael A REED Marion REES Michael John STOTT Andrew J. TEUTEN Rachel M. THORNLEY Janis Florence VOLCKMAN
ADDRESS	Glaxo Wellcome plc Glaxo Wellcome House Berkeley Avenue Greenford Middlesex UB6 ONN Great Britain

Propagation Method

The present invention relates to a method for the propagation of lytic organisms, and its use for the production of lytic organisms and proteins.

A number of different organisms can be grown in tissue culture for use in research studies or vaccine production. However, the titres obtained in tissue culture, although suitable for research purposes, are often too low to be commercially useful in the vaccine industry, so bulk preparations of vaccines (e.g. flu vaccine) are often cultured in chicken eggs. This system provides very high titres of virus but requires a large amount of processing and quality control of product before release. The process is very labour intensive and has problems with continuity of supply and reproducibility of product from batch to batch. Hence there is a need for large scale, reproducible, high titre production of organisms.

Another area that feels a critical need for large scale high titre reliable virus production is gene therapy. Recombinant adeno-associated virus vectors have recently been shown to be efficient, non-immunogenic and persistent vectors for gene therapy. However, current technologies are unable to produce the amounts of recombinant viruses needed for this field to move forward (Linden, R.M and Woo, S.L.C. Nature Medicine **5**(1) 1999 pp 21 - 22.)

The culture of lytic organisms is particularly difficult as these quickly destroy the cell population they are grown in. The insect virus baculovirus is a lytic organism which can be used for the production of proteins. These viruses can be engineered for recombinant protein expression (Gruenwald, S. and Heitz, J. Baculovirus expression vector system: Procedures and methods manual. Pharmingen). They are generally grown in spinner culture, in which maximum infection and hence maximum protein production is reached after two days. A longer term culture of baculovirus would produce greater amounts of protein, and be extremely useful but, until now, has not been possible.

The present invention provides a method for the propagation of lytic organisms which comprises the infection of the cells of a stable cell line within a hollow fibre

bioreactor with a lytic organism, wherein after said infection, said organism multiplies within the cell line and can be harvested, characterised in that the cell line can survive for at least ten days after infection.

The invention further provides a method as described above, wherein the lytic organism contains nucleic acid encoding a protein of interest, and after said infection this protein is expressed by the cells and can be harvested.

In a preferred embodiment the cell line can survive for at least twelve days after infection, preferably 15 days, more preferably 20 days. Particularly preferred is when the cell line can survive for 25, 30, 40, 50 or 60 days after infection with the lytic organism. For the duration of the time that the cell line survives, the lytic organism is produced and can be harvested. Also, if the lytic organism contains nucleic acid encoding a protein of interest, this is expressed as long as the cell line survives, and harvests can be taken during this time.

Further provided is any of the methods as described above which further comprises the step of harvesting of either the lytic organism or the expressed protein. This step comprises removal of the lytic organism or the expressed protein from the bioreactor by any means. Preferably, the lytic organism or the expressed product is removed by flushing of the medium from the bioreactor.

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

A Hollow fibre bioreactor as used in the method of the invention is a continuous perfusion system. One example of a typical hollow fibre bioreactor is BR1910 which is available from UNISYN® Technologies. The reactor employs multiple fibres consisting of semipermeable polymeric membranes. The membranes may be made of different materials. Preferably the semipermeable polymeric membranes are cellulose acetate.

The fibres provide a large surface area for cell contact, in a relatively compact system. The cell line grows outside the fibres in the extracapillary space (ECS), and fresh medium is passed through the fibres in the intracapillary space (ICS). Typically, small molecular weight cellular nutrients and metabolic waste products can pass between the ICS and the ECS whilst cells and lytic organism or expressed protein products are confined to the ECS. A "feed" or "factor addition" port is used to introduce the lytic organism, or to feed large molecular weight nutrients into the ECS. A harvest port is used to remove the medium containing the harvest from the ECS.

Infection comprises introducing the lytic organism into the ECS so that it may infect the cells of the stable cell line. Preferably, the lytic organism is introduced as a suspension, or more preferably as a seeder culture. A seeder culture comprises a small number of cells which are of the same cell line as is within the bioreactor. The cells of the seeder culture, however, additionally comprise the lytic organism. The seeder culture is introduced into the ECS, and the cells of the seeder culture lyse, causing the lytic organism to be released into the ECS.

The term lytic organism as used throughout the specification and claims is intended to encompass any organism, whether in its natural state, or which has been modified genetically or in any other way, which is capable of infecting a cell and subsequently causing it to lyse. The lytic organism is destructive to the cells it infects. Multiplication of the organism within the cell leads to lysis and death of the cell and release of the organism into the surrounding medium. In the situation where the lytic organism contains nucleic acid encoding a protein of interest, this protein is expressed by the cell before lysis.

Typical lytic organisms encompass eukaryotes, prokaryotes, for example mycobacteria, and viruses, for example baculovirus; herpesviruses such as HSV-1, HSV-2, VZV; parvoviruses such as B19, AAV-2; arboviruses such as rubella, yellow fever, sandfly fever, lymphocytic choriomeningitis, Getah arbovirus, and including arenaviruses, buyaviruses, flaviviruses, togaviruses; diarrhoea viruses such as astroviruses, caliciviruses, reoviruses, rotaviruses, porcine epidemic diarrhoea virus; polyoma viruses such as SV40; picornaviruses such as poliovirus, enterovirus e.g. echovirus, rhinovirus; papillomaviruses such

as human papillomavirus and bovine papillomavirus; filoviruses such as marburg virus, ebola virus; poxviruses such as smallpox, copox, variola virus, vaccinia; adenoviruses; orthomyxoviruses such as influenzavirus A, influenzavirus B, influenzavirus C, thogoto-like viruses; paramyxoviruses such as Parainfluenzavirus 1-4, senai virus, mumps, Newcastle disease virus; orbiviruses for example rheoviruses, bluetongue virus; iridoviruses such as African seine fever virus, and Moriblliviruses such as measlesvirus, rinderpest virus, canine distemper virus, pneumovirus, repsiratory syncytial virus; also African swine fever virus, bluetongue virus, bovine leucosi, echovirus and revirus. Preferably the lytic organism is a virus, more preferably it is adenovirus or a baculovirus.

In some situations, a modified lytic organism will be preferable to a lytic organism in its natural state, for example the use of baculovirus containing nucleic acid encoding a protein of interest to achieve expression of that protein, or in another case, when producing organisms for vaccines or gene therapy it may be desirable to use a lytic organism which has been modified to be replication defective, or attenuated in some way to make it less harmful to humans.

The term stable cell line as referred to above is intended to encompass all cell lines that are capable of growing in the extra capillary space of the bioreactor to establish a population of 10^6 cells per ml. At the point of infection with the lytic organism, the cells are preferably confluent. By confluent is meant that when examining the cells by eye, they have grown to cover the majority of the surface area. At the point of infection with the lytic organism, the cell density will preferably be 10^6 cells per ml. More preferably the cell density will be 10^7 cells per ml. Particularly preferred is when the cell density at the point of infection with the lytic organism is 10^8 or 10^9 cells per ml. It is thought to be due to the high cell density used in the method of the invention that the cell line can survive for so long after infection of the lytic organism. The cells are postulated to form a matrix, which prevents all the cells within the matrix becoming infected immediately, and hence allows the cell line to survive and proliferate for longer.

Such cell lines include, for example, stable higher eukaryotic cell lines such as mammalian cell lines or insect cell lines, lower eukaryotic cell lines such as yeast cell lines or prokaryotic cell lines such as bacterial cell lines. The particular cell line chosen will vary depending on whether the desired product is a lytic organism, or the expression product of nucleic acid contained within the lytic organism, and also the lytic organism used for infection. In the situation where the lytic organism contains nucleic acid encoding a protein of interest, and this protein is produced within the cell line, the cell line selected will preferably be one which allows for the correct post-translational modification of that protein to occur. Particular examples of cells which can be used are vero cells which are susceptible to many typical lytic organisms as described above, for example ~~adenovirus 12, African swine fever virus, arbovirus, bluetongue virus, echovirus,~~ Getah arbovirus, herpes simplex virus, influenza virus, orbivirus, orthomyxovirus, paramyxovirus, poliovirus 3, porcine epidemic diarrhea virus, rheovirus and rubella; Hep-2C cells which are susceptible to, for example arbovirus and measles virus; PerC6 cells which are susceptible to, for example adenovirus; SF9 or Tni cells which are susceptible to, for example baculovirus, and human macrophage cell lines such as THP-1 which is susceptible to, for example mycobacteria such as tuberculosis or BCG.

It will be understood by a person skilled in the art that the conditions within the bioreactor may vary depending on which cell line is used, which lytic organism is used for infection, and whether the desired product is a lytic organism, or the expression product of nucleic acid contained within a lytic organism. Particularly, the medium used will vary depending on the cell line. For example when using a mammalian cell line such as Vero cells, THP-1 cells or PerC6 cells, the medium is preferably DMEM, RPMI, Ham's F12, or similar medium or a custom derived medium specific for each cell line. When using an insect cell line such as Tni or SF-9, the medium is preferably SF900II, TC100 or similar insect cell medium. Supplements, for example antibiotics, feotal calf serum (FCS), glutamax™, δ-Aminolaevulinic Acid, ferric citrate, nicotinic acid, riboflavin and hemin chloride may be added when necessary, for example when producing a specific protein.

The nucleic acid referred to in the specification and the claims as being contained within the lytic organism may be in the form of RNA or DNA, for example cDNA, genomic DNA, or synthetic DNA. The nucleic acid can be, for example, a whole gene, the coding region of a gene, a desired fragment of a gene or a wholly synthetic nucleic acid, depending on the protein required.

The term "protein" as used throughout the specification and the claims is also intended to include within its meaning shorter peptide or polypeptide sequences as well as complete proteins. The method of the invention is useful for the production of full length proteins, including but not limited to, receptors, cytokines, growth factors, adhesion molecules for example ICAM, and enzymes, for example cytochrome p450 reductase or tyrosine kinase. The method of the invention can also be used to produce truncated proteins, chimeric proteins or fragments of proteins for example an antigenic portion, or a ligand binding site. Wholly synthetic polypeptide sequences of any desired length can also be produced by this method. Any of the proteins which can be produced by the method of the invention can also be produced in modified form for example by the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence

In another aspect of the invention, in the method described above, after harvest the cell line is allowed to re-populate the bioreactor preferably with the introduction of fresh medium, but without introduction of any further cells into the bioreactor, and at least one subsequent harvest may be taken, with the cell line being allowed to re-populate the bioreactor after each harvest. Preferably at least two subsequent harvests may be taken, more preferably at least three, four or five subsequent harvests may be taken. This aspect applies both to the situation where the lytic organism itself is harvested, and where the lytic organism contains nucleic acid encoding a protein of interest, and the protein is harvested.

The repeated harvesting of product and repopulation of cells is thought to occur in the following way. Following addition of the lytic organism into the ECS of the hollow fibre bioreactor containing the stable cell line, it seems that the lytic organism will infect some of the cells of that cell line, where it will multiply and

cause subsequent lysis of these cells, releasing the lytic organism produced within that cell into the bioreactor. This will then go to infect more cells, multiply within them and cause their lysis. In this way the organism multiplies, and the cell line is gradually caused to lyse and die. However, due to the high cell density used in the method of the invention, it appears that the lytic organism will not undergo this cycle sufficiently frequently to infect all of the cells of the cell line before harvest. At harvest, most of the lytic organisms are removed. After harvest the bioreactor contains cells infected with the lytic organism which have not yet lysed and healthy, uninfected cells. At this point, if fresh medium is added to the bioreactor, the cells remaining within the bioreactor continue to divide and grow, and repopulate the bioreactor, providing a fresh population of cells for the cycle of infection and lysis to continue. In the situation where the lytic organism contains a nucleic acid encoding a protein of interest, the new population of cells will express this protein, which can be harvested. Thus in this aspect, the invention provides a method for cyclical production of lytic organisms or a desired protein.

In another aspect of the invention is provided a method for studying the effects of molecules on a lytic organism which comprises infection of the cells of a stable cell line within a hollow fibre bioreactor wherein after said infection, varying amounts of said molecules may be added, and their effects on the lytic organism measured, characterised in that said cell line can survive for at least ten days after infection. Thus is provided, for example, a means of testing the effect of drugs on the infectivity or life cycle of an organism, or a means of testing the pharmacokinetics or pharmacodynamics of drugs during an infection caused by a lytic organism. As the present invention provides longer term survival of the cell line after infection than existing systems, drug interactions and effects can be studied over a longer time period, opening the possibility of using the system for looking at the effect of molecules on chronic infections for example Herpes Simplex Virus , Papilloma virus, or mycobacterial infections.

Brief description of the figures:

Figure 1: Adenovirus production in a hollow fibre bioreactor. This figure shows both the long term production before harvest (21 days) and the repopulation of the bioreactor, leading to further virus growth after harvest, up to 60 days.

Figure 2: Production of cytochrome p450 reductase after infection with baculovirus containing DNA encoding this protein. The OD450 (measured by Virus specific ELISA) is proportional to the viral titre, and the solid bars show the protein levels harvested in activity units. This graph indicates that long term production is possible with this method, with significant amounts of protein being harvested at 14 days after infection, a much longer term than has been possible before. It can also be clearly seen from the graph that cyclical production of the protein is possible, with a subsequent increase both in virus titre and protein production after the first harvest.

Figure 3: Production of the mycobacterium BCG within THP-1 cells in a hollow fibre bioreactor. Samples were taken at 4, 8, 14 and 21 days after infection. The large circles are macrophage cells. This figure indicates long term survival of the cell line and production of BCG.

Examples

Example 1: Cyclical production of mammalian virus.

For adenovirus specifically, the cells used are PerC6 cells (introgene). However, this method is applicable to all mammalian cell lines and mammalian viruses described in the specification.

Cells were grown in 6 x 175sq.cm. flasks in DMEM (Life Technologies) supplemented with 2mM glutamax ™(Life Technologies) , 5% FCS (Life Technologies).
The cells were scraped off or aspirated off at confluence and transferred to the extracapillary space (ECS) of a BR1910 (Unisyn Technologies) hollow fibre bioreactor (19sq.ft surface area, 10kD molecular weight cut off, membrane material cellulose acetate) . Routinely around 10e8 cells are inoculated into the ECS of each BR1910.

The intracapillary space (ICS) was constantly perfused with DMEM {Life Technologies}supplemented with 2mM glutamax ™(Life Technologies). at a recirculation rate of 100 - 500 ml/min. The re-circulation rate is low at the time of inoculation and the rate increases with time in culture as the cell density within the bioreactor increases.

The recirculating medium was held in a reservoir containing 1.0 litre, with a continuous medium change via a feed and harvest skimmer system of 0.8 litres/day to 2.0 litre /day. The feed rate is set low at inoculation and increases with cell density over time.

The medium was oxygenated by an OXY10 (Unisyn Technologies) oxygenation cartridge (10sq.ft. surface area, pore size 0.2um, membrane material ~~microporous polyethylene fibre) perfused with 95% air/ 5% CO2 at a pressure of~~ 2 psi.

Cells were then grown until the bioreactor was deemed to be confluent by eye (usually between 20 and 80 days). The cell mass in the ECS received regular feeding by exchanging 50ml of the spent ECS medium with 50ml fresh medium consisting of DMEM(Life Technologies) /5%FCS(Life Technologies) /2mMglutamax (Life Technologies) at intervals not less than weekly. Fresh medium is introduced and spent medium removed via the feed and harvest ports in the bioreactor.

Virus was inoculated into the ECS via one of the feed and harvest ports, and the infection allowed to continue until a suitable harvest point. In the first instance, samples were taken to monitor progress of the infection, and harvests were taken subsequently at 7 - 10 day intervals. The ECS was extensively harvested by flushing with 2 x 50ml aliquots of culture medium and by passing medium across the membrane - ie from ICS to ECS, and the product stored for subsequent analysis.

The cell mass in the ECS was then allowed to regenerate by feeding with DMEM (Life Technologies) supplemented with 2mM glutamax ™ (Life Technologies), 5% FCS (Life Technologies), and virus titre was monitored to determine the "recovery " phase. See figure 1 for results.

Example 2: Production of a protein of interest from a baculovirus containing DNA encoding that protein.

The cells were SF9 cells, at a concentration of around 10^6 cells/ml. The cells were maintained using standard methodologies, with a passage frequency of 3-4 days. The medium used is a specialist insect cell culture medium, SF900 II (supplemented), obtained from Life Technologies (Cat. No.041-94322). This is also made up to contain Ultra Low IgG Foetal Bovine Serum (U.S.) at a concentration of 10%, that was also obtained from Life Technologies (Cal.No.10118-164).

In order to inoculate the bioreactor, it is necessary to produce around 10e8 cells. Thus 4 x 225cm^2 tissue culture flasks are inoculated to produce these cells. Inoculation of the cells is carried out via the factor addition port of the bioreactor, into the ECS. Excess medium is removed via the harvest port., Once the cells are inoculated, the feed and harvest ports are closed, and the re-circulation and feed/waste pumps are switched on.

The cells are left for 7-21 days in order to adapt to their new situation, before infection with virus occurs. This period can be longer if required, as the cells can be maintained in the bioreactor indefinitely. During this period air should be run through the oxygenator to saturate the medium with oxygen. The pumps should be running at between 0.8 and 2 litres/day for the feed and waste skimmer pump(increasing with cell density), and the re-circulation pump at around 100 - 500ml/min(also increasing with cell density).The temperature should be a constant 27 ^{0}C.

The infection with virus is carried out at a multiplicity of infection (MOI) estimated to be 1.0. Thus the volume of virus suspension that should be added will depend on the concentration of virus (in PFU/ml) and the number of cells present in the bioreactor.

Addition of the virus is done through the factor addition port, along with SF900 and the required volume of any necessary supplements (see example 4). Some of the medium forced out of the harvest port is retained to give a time 0 sample. The bioreactor is then sampled at day 2, 4 and 6, and at subsequent weekly intervals to monitor the infection. Initially samples should be of 50ml,taken while adding fresh medium, FCS and other supplements. Larger harvests are taken as detailed in example 1. These large samples are then carried out on a weekly basis to monitor long term virus and protein production. As in example 1, this system can be operated in a cyclical manner by allowing the cell mass to regenerate by feeding with fresh medium, and any necessary supplements.

Example 3: Production of Cytochrome p450 from baculovirus

This is essentially as described in example 3. However, due to the abnormally high levels of P450 and P450 reductase expression that should occur in the system if these proteins are being produced, the cells will require higher than normal levels of certain nutrients, in order to maintain their intracellular reservoirs. To this end the cells are supplied with certain supplements:

δ-Aminolaevulinic Acid (δALA) at 0.1mM,
Ferric Citrate at 0.1mM,
Nicotinic Acid at 0.05μM,
Riboflavin at 0.01μM,
Hemin Chloride at 2mg/l solution.

All these supplements were obtained from Sigma Biosciences, and made up as stock solutions using SF900 medium as before. The exceptions are ferric citrate, which should be dissolved in boiling water, and hemin chloride, which should be dissolved in 0.1M sodium hydroxide after which an equal volume of water should be added. These supplements are used in the concentrations indicated, and should be added to both the bioreactor ECS and the feed bottle/bag in order to maintain the required concentration throughout the system. Note that these supplements are only required once the cells have been infected with the baculovirus and if the baculovirus insert contains the P450 and reductase genes. Inserts not producing P450 will not require the supplements. See figure 2 for results.

Example 4: Production of BCG

THP-1 cells were grown in 6 x 175sq.cm. flasks in DMEM (Life Technologies) supplemented with 2mM glutamax ™(Life Technologies) , 5% FCS (Life Technologies).

The cells were scraped off or aspirated off at confluence and transferred to the extracapillary space (ECS) of a BR1910 (Unisyn Technologies) hollow fibre bioreactor (19sq.ft surface area, 10kD molecular weight cut off, membrane material cellulose acetate) . Routinely around $10e^8$ cells are inoculated into the ECS of each BR1910.

The intracapillary space (ICS) was constantly perfused with DMEM {Life Technologies}supplemented with 2mM glutamax ™(Life Technologies). at a recirculation rate of 100 - 500 ml/min. The re-circulation rate is low at the time of inoculation and the rate increases with time in culture as the cell density within the bioreactor increases.

The recirculating medium was held in a reservoir containing 1.0 litre, with a continuous medium change via a feed and harvest skimmer system of 0.8 litres/day to 2.0 litre /day. The feed rate is set low at inoculation and increases with cell density over time.

The medium was oxygenated by an OXY10 (Unisyn Technologies) oxygenation cartridge (10sq.ft. surface area, pore size 0.2um, membrane material microporous polyethylene fibre) perfused with 95% air/ 5% CO2 at a pressure of 2 psi. Cells were then grown for three weeks. The cell mass in the ECS received regular feeding by exchanging 50ml of the spent ECS medium with 50ml fresh medium consisting of DMEM(Life Technologies) /5%FCS(Life Technologies) /2mMglutamax (Life Technologies) at intervals not less than weekly. Fresh medium is introduced and spent medium removed via the feed and harvest ports in the bioreactor.

The cell mass at confluence was treated with phorbol myristic acid (PMA) at 5ng/ml to induce differentiation. 24 hours after differentiation, 10e8 cfu of BCG were added to the bioreactor, and infection monitored by sampling at time intervals over 30 days. Final harvests were by flushing the ECS with medium, flushing medium across the membrane, flushing out the ECS in 2% SDS,and finally 5mM arabinose.

Samples were retained for analysis. See figure 3 for results of infection.

The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process or use claims and may include, by way of example and without limitation, one or more of the following claims:

Claims

1. A method for the propagation of lytic organisms which comprises the infection of the cells of a stable cell line within a hollow fibre bioreactor with a lytic organism, wherein after said infection, said organism multiplies within the cells and can be harvested, characterised in that the cell line can survive for at least ten days after said infection.

2. The method of claim 1 which further comprises the step of harvesting the lytic organism, by removal of said organism from the bioreactor.

---

3. A method according to claim 1, wherein the lytic organism contains nucleic acid encoding a protein of interest, and after said infection this protein is expressed by the cells and can be harvested.

4. The method of claim 3 which further comprises the step of harvesting the protein of interest by removal of said protein from the bioreactor.

5. A method according to any preceeding claim, characterised in that the cell line can survive for at least 15 days after infection.

6. A method according to any preceeding claim, characterised in that the cell line can survive for at least 20 days after infection.

7. A method according to any preceeding claim, wherein after harvest, the cell line is allowed to re-populate the bioreactor, and at least one subsequent harvest may be taken, with the cell line being able to re-populate the bioreactor after each harvest.

8. A method according to claim 7 wherein at least 2 subsequent harvests may be taken.

9. A method according to claim 7 wherein at least 3 subsequent harvests may be taken.

10. A method according to any one of the preceeding claims wherein said lytic organism is a virus.

11. A method according to claim 10 wherein said virus is a baculovirus.

12. A method according to claim 10 wherein said virus is an adenovirus.

13. A method for studying the effects of molecules on a lytic organism which comprises the infection of the cells of a stable cell line within a hollow fibre bioreactor, wherein after said infection, varying amounts of said molecules ~~may be added, and their effects on the lytic organism measured,~~ characterised in that said cell line can survive for at least ten days after infection.

14. An apparatus for carrying out the method according to any preceeding claim comprising a hollow fibre bioreactor containing a stable cell line capable of surviving for at least ten days after infection of the cells of said cell line with a lytic organism.

Figure 1

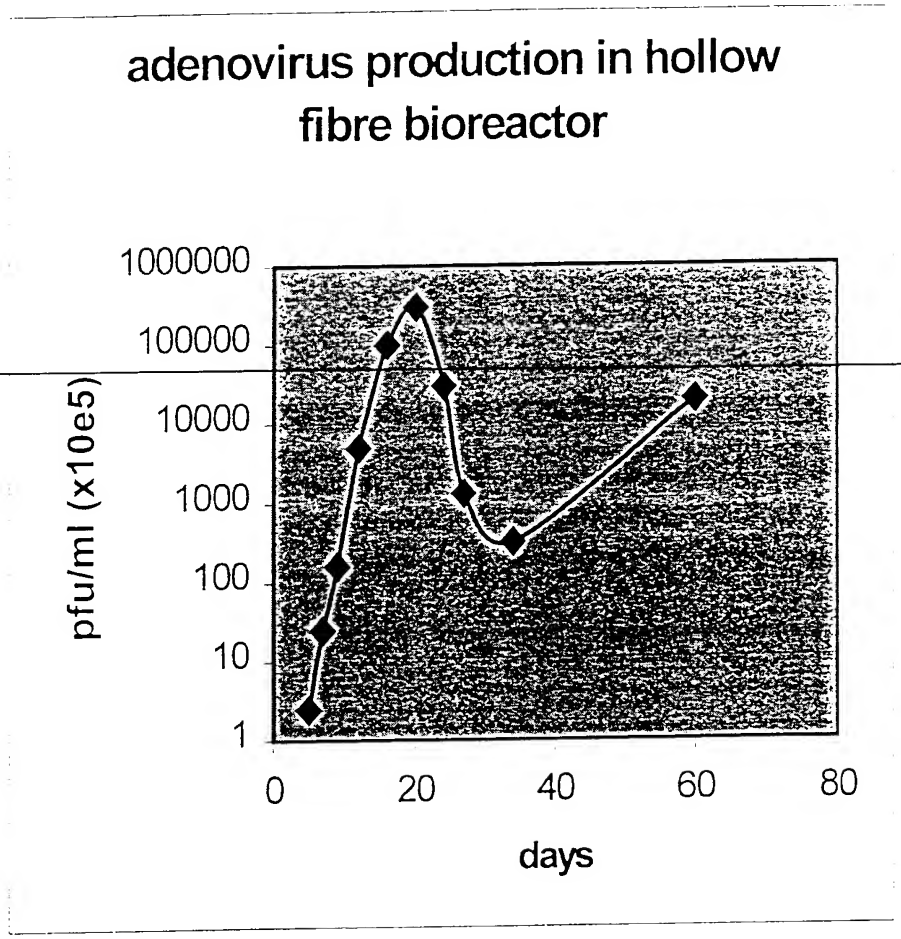
adenovirus production in hollow fibre bioreactor
pfu/ml (x10e5)
1000000
100000
10000
1000
100
10
1
0
20
40
60
80
days

Figure 2.

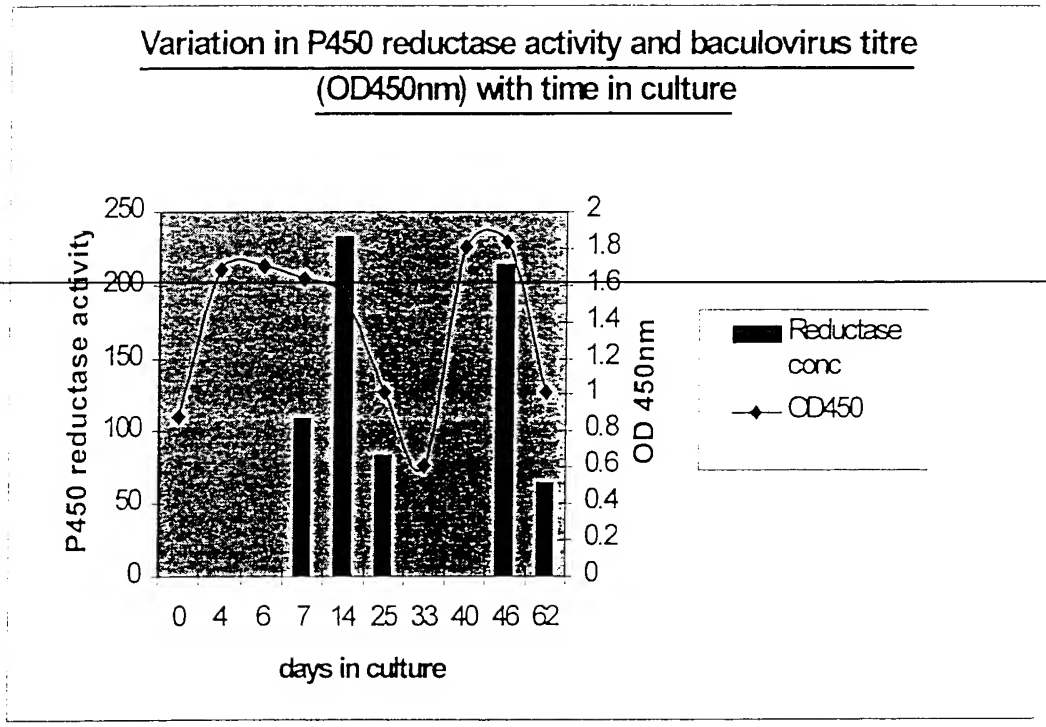
Variation in P450 reductase activity and baculovirus titre (OD450nm) with time in culture
P450 reductase activity
250
200
150
100
50
0
OD 450nm
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 4 6 7 14 25 33 40 46 62
days in culture
Reductase conc
OD450

Figure 3

Progress of BCG infection in THP-1 cells in a bioreactor